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ION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Assessment of efficacy of activated charcoal for treatment of acute T-2 toxin poisoning	5. TYPE OF REPORT & PERIOD COVERED DTIC FILE COPY
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Robert F. Fricke and Juan M. Jorge	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Research Institute of Infectious Diseases, SGRD-UIS-D Fort Detrick, Frederick, MD 21701-5011	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command	12. REPORT DATE 14 November 1986
	13. NUMBER OF PAGES 10 + 4 Figures
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report)
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution unlimited - approved for public release

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

To be published in Journal of Toxicology - Clinical Toxicology

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

T-2 toxin is a fungal metabolite which can cause death or illness upon ingestion. As a potential antidote, activated charcoal was assessed for efficacy in decreasing the lethality of both oral and parenteral exposure to T-2 toxin. In vitro binding studies, using the Langmuir adsorption isotherm, showed that activated charcoal had a maximal binding capacity of 0.48 mg toxin/mg charcoal and a dissociation constant of 0.078 mg charcoal/l. In vivo, orally administered, activated charcoal was assessed for treatment of acute oral or parenteral exposure to T-2 toxin in mice. After oral toxin administration (5 mg/kg)

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untreated mice showed only 6% survival after 72 hr. Charcoal treatment (7 g/kg, po) either immediately or 1 hr after toxin exposure resulted in significant improvement in survival, with values of 100% and 75%, respectively. After parenteral toxin exposure (2.8 mg/kg, sc), untreated and charcoal-treated (7 g/kg, po) mice showed 50% and 90% survival, respectively, after 72 hr. LD_{50} value for T-2 toxin, determined at 96 hr after intoxication, increased significantly from 2 mg/kg for untreated controls to 4.5 mg/kg for activated charcoal treatment.

ASSESSMENT OF EFFICACY OF ACTIVATED CHARCOAL FOR TREATMENT OF ACUTE T-2 TOXIN POISONING[1]

Robert F. Fricke[2], Ph.D. and Juan M. Jorge
Pathophysiology Division
U.S. Army Medical Research
Institute of Infectious Diseases
Ft. Detrick, Frederick, Maryland 21701-5011

ABSTRACT

T-2 toxin is a fungal metabolite which can cause death or illness upon ingestion. As a potential antidote, activated charcoal was assessed for efficacy in decreasing the lethality of both oral and parenteral exposure to T-2 toxin. In vitro binding studies, using the Langmuir adsorption isotherm, showed that activated charcoal had a maximal binding capacity of 0.48 mg toxin/mg charcoal and a dissociation constant of 0.078 mg charcoal/l. In vivo, orally administered, activated charcoal was assessed for treatment of acute oral or parenteral exposure to T-2 toxin in mice. After oral toxin administration (5 mg/kg), untreated mice showed only 6% survival after 72 hr. Charcoal treatment (7 g/kg, po) either immediately or 1 hr after toxin exposure resulted in significant improvement in survival, with values of 100% and 75%, respectively. After parenteral toxin exposure (2.8 mg/kg,sc), untreated and charcoal-treated (7 g/kg, po) mice showed 50% and 90% survival, respectively, after 72 hr. LD_{50} value for T-2 toxin, determined at 96 hr after intoxication, increased significantly from 2 mg/kg for untreated controls to 4.5 mg/kg for activated charcoal treatment.

INTRODUCTION

T-2 toxin is a toxic secondary metabolite produced by a variety of Fusarium fungi [1]. This trichothecene mycotoxin is highly toxic and has produced illness and death in humans and livestock after ingestion of food or grain contaminated with fungal toxins. Oral exposure to T-2 toxin has profound effects on rapidly dividing tissues [2]. Thus, the spleen, testes, thymus, bone marrow, gastrointestinal tract, and hair follicles all show significant necrosis [3,4].

One of the most consistent and well-defined effects of T-2 toxin in several animal species is the dramatic cytotoxic changes in the gastrointestinal tract [2,3,4]. After parenteral [3], topical [5], or oral [2] exposure, there is severe and prolonged diarrhea [6], accompanied by increased intestinal permeability [7].

The severe gastrointestinal effects of T-2 toxin may be a result of the preferential excretion of T-2 toxin metabolites via the bile into the intestine [8]. Activated charcoal might be effective in decreasing lethality by binding toxin metabolites in the gastrointestinal tract. The toxic compounds would therefore remain in the gastrointestinal tract, decreasing the reabsorption via enterohepatic recirculation. In the studies presented here, efficacy of activated charcoal for treatment of both oral and parenteral toxin exposure was assessed. cont'd pg

MATERIALS AND METHODS

In Vitro Experiments

In vitro adsorption experiments were performed to determine the equilibrium binding and dissociation constants for T-2 toxin and activated charcoal (Amoco AX-21, Anderson Development Co., Adrian, Mich.). The charcoal used in these experiments was highly activated and has been shown to be superior to other forms of activated charcoal for treatment of a variety of intoxications [9].

A stock solution of T-2 toxin, >99% purity (Wyco-Labs, Chesterfield, Mo.), was prepared in 100% ethanol at a concentration of 25 mg/ml. The stock was diluted with ethanol to final concentrations of 1.31, 0.61, and 1.23 mg ml. Constant amounts of [^{3}H]T-2 toxin (New England Nuclear, Boston, Mass.) were added to each of the above dilutions to give final specific activities of 75, 36, and 18 μCi/mg toxin, respectively. Aliquots of labeled T-2 toxin were mixed with suspensions containing 6.25 to 200 mg charcoal per ml of sodium phosphate buffer (0.05 mM, pH 7.4). After 30-min incubation at room temperature, free and bound toxin were separated by centrifugation (Fisher Microfuge, 3 min, maximum speed). Radioactivity was determined in an aliquot of the supernatant fraction.

By using the specific radioactivities of T-2 toxin, the amount of free, C (mg toxin/l), and bound, Q (mg toxin/mg charcoal), toxin were calculated. The data were analyzed by the equation for the Langmuir isotherm [10]:

$$\frac{C}{Q} = \frac{1}{Q_m K} + \frac{C}{Q_m}$$

The values of K, the equilibrium binding constant, and Q_m, the maximum binding capacity (mg toxin/mg charcoal), were derived after linear regression analysis of the above equation.

In Vivo Experiments

Male mice (Swiss ICR), weighing 21-24 g, were maintained at controlled temperature (21-22°C) in wire-bottom cages and allowed feed and water ad libitum. The stock toxin solution was diluted with propylene glycol:ethanol (90:10) and 100 μl was administered either subcutaneously or orally to 16-hr fasted mice. Activated charcoal was suspended in distilled water and administered by gavage in a total volume of 500 μl.

The number of surviving mice was determined at different times after administration of T-2 toxin. Data were analyzed for statistical significance using the Fisher's exact test [11]. LD_{50} values for T-2 toxin were calculated by the probit regression analysis based on best fit common slope [12]. Statistical significance of the LD_{50} values was determined by least significant difference analysis on the pooled variance of the LD_{50} values [13].

RESULTS

In Vitro Experiments

Langmuir isotherm (Figure 1) was used to calculate the maximum binding capacity, Q_m, and the dissociation constant, K, for the adsorption of T-2 toxin onto activated charcoal. The calculated values (± S.D.) for Q_m and K were 0.48 ± 0.136 mg toxin/mg charcoal and 0.078 ± 0.0197 mg charcoal/l, respectively.

In Vivo Experiments

Efficacy of orally administered charcoal for treatment of oral toxin exposure was assessed in mice. Mice were either untreated or treated with activated charcoal at the same time as or 1 hr after challenge with T-2 toxin (5 mg/kg), po). The number of surviving mice was determined over a 72-hr time course (Figure 2). The percent survival of mice in the untreated group declined steadily throughout the observation period, reaching a value of 6% after 72 hr. All of the mice in the group treated immediately with activated charcoal were protected against the lethal effects of T-2 toxin. If treatment was delayed for 1 hr, the percent survival was lower than that of the group treated immediately. The percent survival of animals in the delayed treatment was, however, significantly higher than in the control group.

Efficacy of activated charcoal for treatment of parenterally administered T-2 toxin was also assessed. Immediately following a subcutaneous injection of T-2 toxin (2.8 mg/kg), mice were either left untreated or treated with activated charcoal (7 g/kg). The number of surviving mice was again determined over 72-hr (Figure 3). The percent of surviving mice in the untreated group was 50%, which was significantly lower than the value of 90% for the treated group.

Because oral charcoal administration was effective in decreasing the lethality of subcutaneous toxin exposure, LD_{50} values for T-2 toxin were determined for untreated and charcoal-treated mice. The LD_{50} values were calculated at 24-hr intervals after toxin injection for a total of 96 hr (Figure 4). At all time points, the LD_{50} values for the charcoal-treated mice were significantly higher than for the untreated controls. LD_{50} values for untreated mice decreased from a 24-hr LD_{50} value of about 3 mg/kg to a value of 2 mg/kg at 96 hr. The charcoal-treated mice, on the other hand, did not show a similar decline in LD_{50} values. Throughout the observation period, the LD_{50} values for charcoal-treated mice remained constant at approximately 4.5 mg/kg.

DISCUSSION

Activated charcoal has been used for the treatment of a wide variety of intoxications. Aflatoxicosis in goats [14] and chickens [15] has been treated successfully with activated charcoal. For treatment of T-2 toxicosis, there are several reports in the literature which suggest that adsorbing agents in the gastrointestinal tract might be effective antidotes. The presence of intestinal roughage effectively altered both the toxicity and excretion of mycotoxins. Animals intoxicated with either T-2 toxin or zearalenone and subsequently fed diets high in crude alfalfa fiber [16,17] or bentonite [18] not only showed increased

survival, but had improved weight gain and feed consumption as well. As the percent of fiber in the diet was increased, there was a proportional increase in the fecal, but not urinary, excretion of toxin [19,20]. These data suggest that other, more effective adsorbing agents, such as activated charcoal, might be effective as well.

Activated charcoal was evaluated for efficacy on treatment of acute T-2 toxicosis in mice. Based upon *in vitro* experiments, T-2 toxin was found to be tightly adsorbed onto activated charcoal, with binding and dissociation constants similar to those reported for nefopam [10]. Since activated charcoal was effective *in vitro*, further *in vivo* experiments were carried out to assess its efficacy in treatment of oral and parenteral toxin exposure. For oral administration of charcoal and toxin, if treatment was initiated immediately after toxin exposure, there was a 100% survival of the mice. If treatment was delayed up to 1 hr, there was still significant, but lower, survival.

Perhaps the more important finding is the protective effect of activated charcoal against parenterally administered toxin. Following subcutaneous toxin administration, oral administration of activated charcoal resulted in significant improvement in the proportion of surviving animals and a significantly higher LD_{50} value for T-2 toxin. Parenteral exposure to T-2 toxin results in both free and conjugated metabolites appearing in the bile [8], which may be reabsorbed via the enterohepatic recirculation. The presence of adsorbing agents in the gastrointestinal tract effectively binds the metabolites, thereby short-circuiting the enterohepatic cycle.

In summary, we found activated charcoal to be an effective antidote for treatment of T-2 toxicosis in mice. Survival after oral or subcutaneous toxin exposure was significantly improved by charcoal treatment.

ACKNOWLEDGMENTS

Thanks are due to Dr. J. G. Pace and Dr. K. A. Mereish for their advice during the writing of this paper, and to Patricia Weicht for preparing the manuscript.

FOOTNOTES

1. In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

   The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

2. To whom correspondence and reprint requests should be sent.

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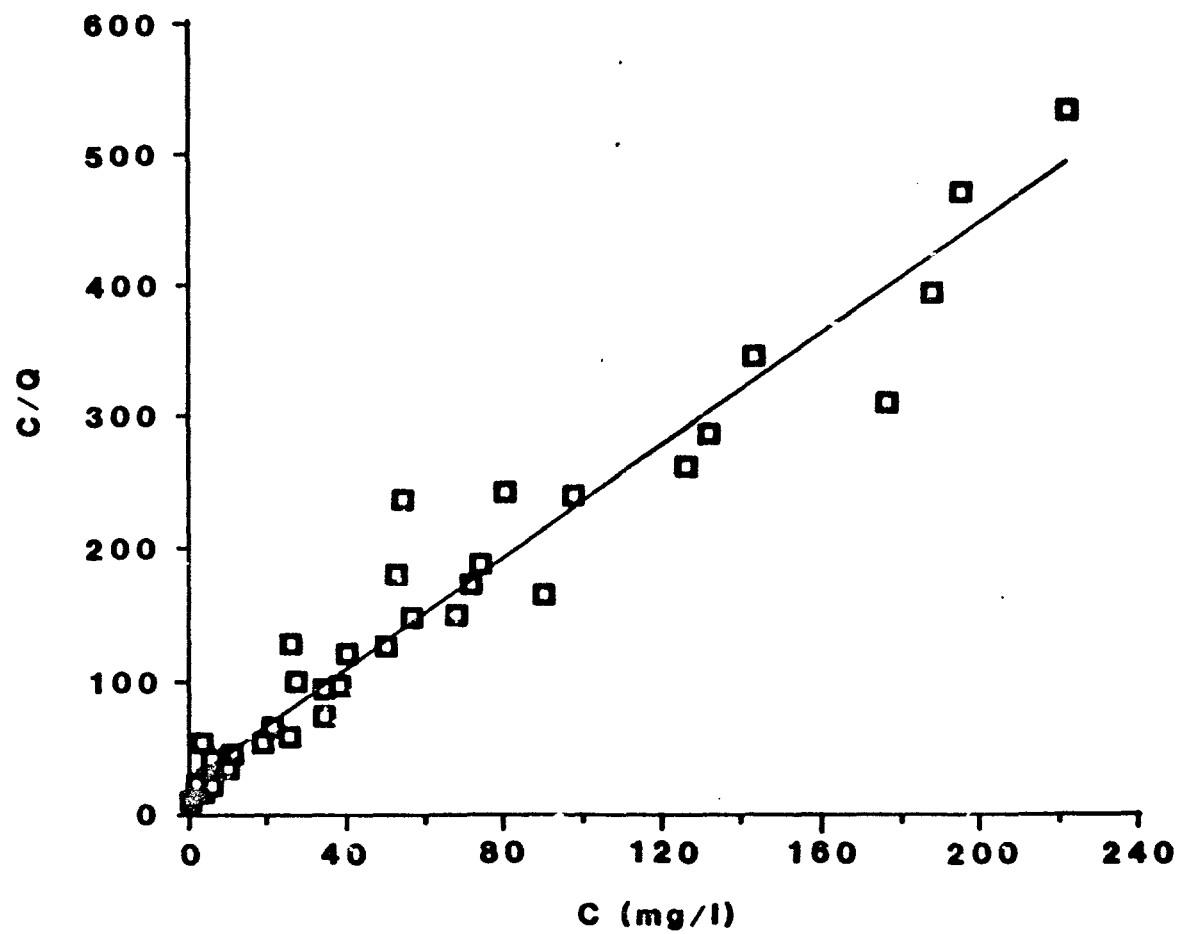
FIGURE 1. Langmuir isotherm for adsorption of T-2 toxin onto activated charcoal.

FIGURE 2. Effect of activated charcoal on the percent survival of mice after oral T-2 toxin exposure. Mice were all gavaged with T-2 toxin (5 mg/kg) and either untreated (closed circles), treated immediately (open circles), or 1 hr later (squares) with activated charcoal (7 g/kg). Significant differences from untreated control values are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

FIGURE 3. Effect of activated charcoal on the percent survival of mice after subcutaneous T-2 toxin exposure. Mice were injected with T-2 toxin (2.8 mg/kg) and either untreated (closed circles) or treated with activated charcoal (7 g/kg) (open cirlces). Significant differences from untreated control values are indicated by * ($p < 0.05$).

FIGURE 4. Effect of activated charcoal on the LD_{50} value for subcutaneously administered T-2 toxin. LD_{50} values were determined for untreated (open bars) or activated charcoal-treated (crosshatched bars) mice at times indicated. Significant differences from untreated control values are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).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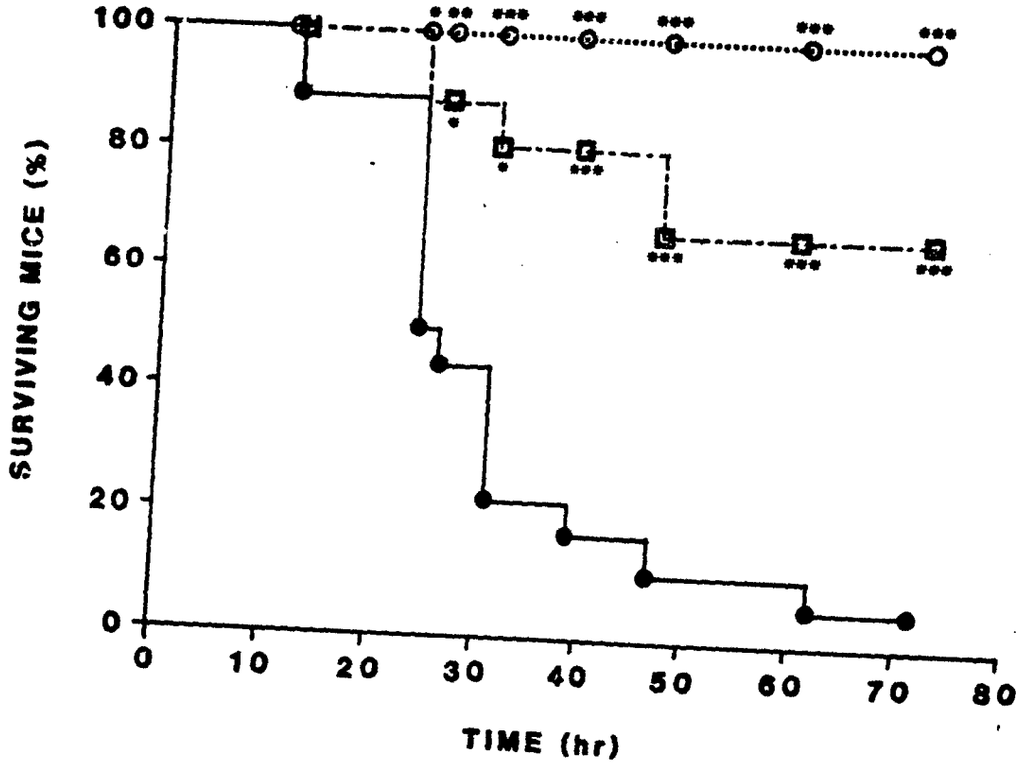
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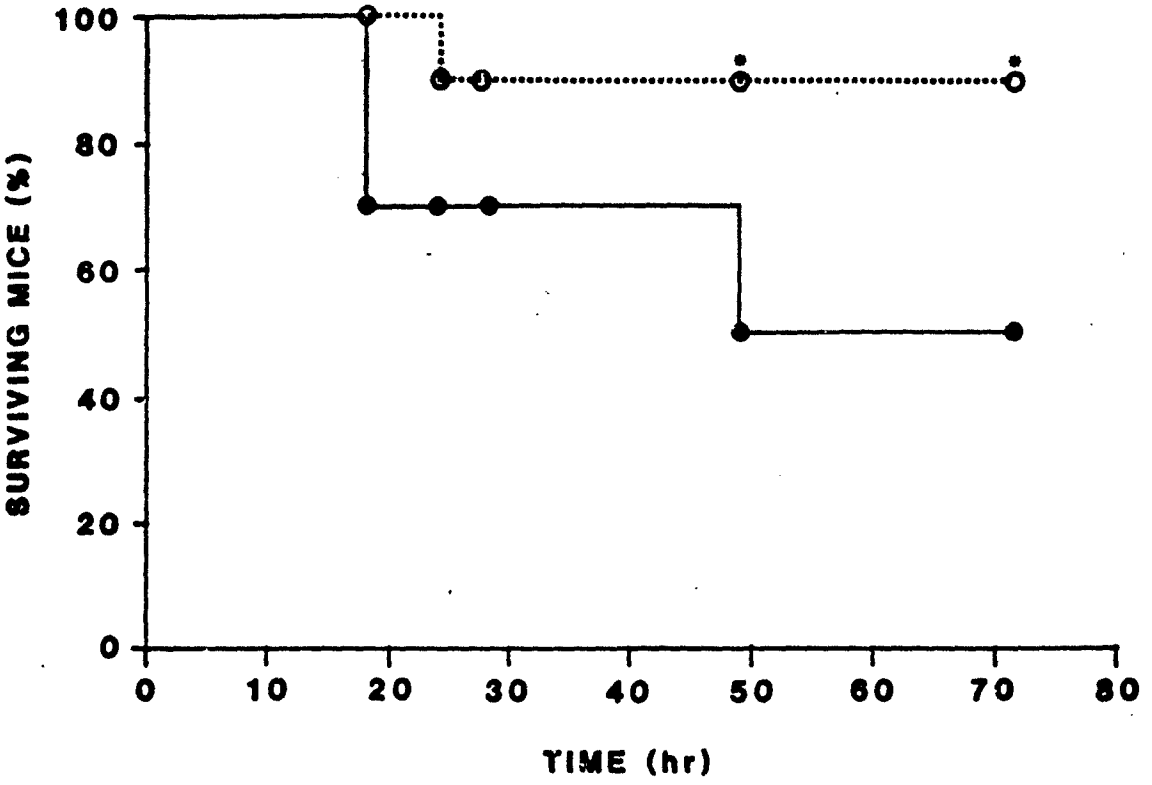
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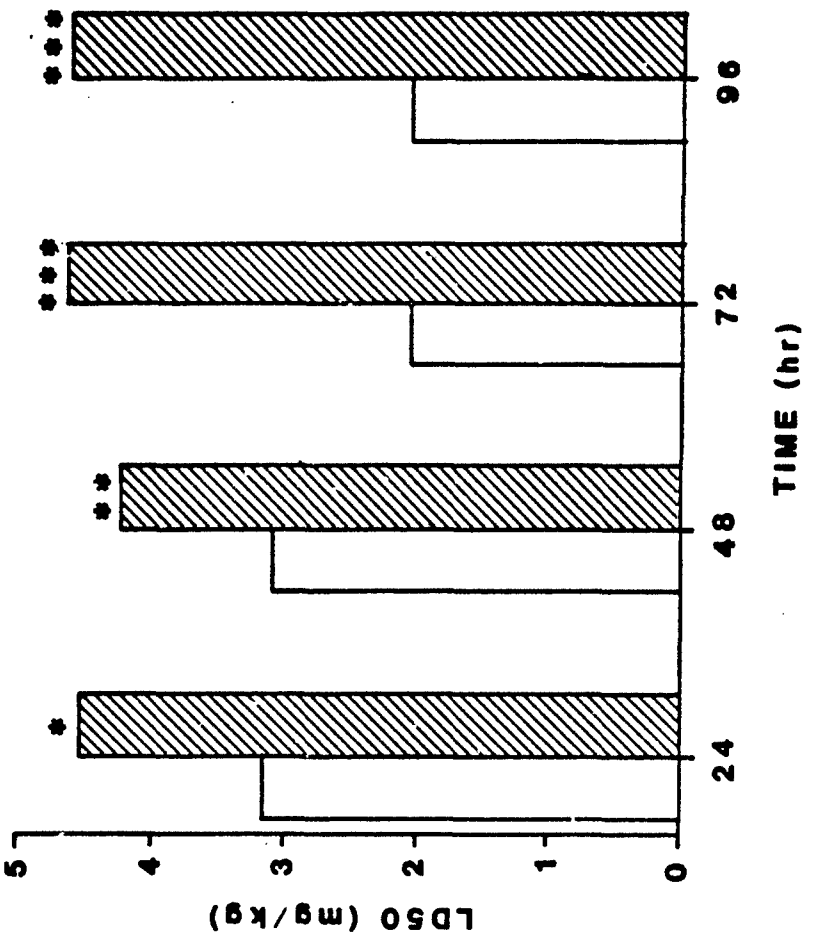
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